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Delta Hepatitis: Molecular Biology and Clinical and Epidemiological Features

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HISTORICAL PERSPECTIVE

Hepatitis delta virus (HDV) was discovered in 1977 by Rizzetto and colleagues while they were studying liver biopsies of patients with hepatitis B surface antigen (HBsAg)-positive chronic liver disease (157). Rizzeto et al. noted that fluorescein-labeled immunoglobulins prepared from a human serum specimen that had been shown to contain antibodies to hepatitis B core antigen (anti-HBc) reacted not only to liver tissues containing HBcAg but also to some tissues devoid of this antigen. Blocking and absorption studies showed that the antibody present in this preparation was not specific for any previously characterized hepatitis B virus (HBV) antigens or cellular antigens. Although this new antigen-antibody system, designated delta, was unrelated to HBV antigens, it was associated with HBV, since the delta antigen (HDAg) could be detected only in those patients who were HBsAg positive. Development of a radioimmunoassay (RIA) and its subsequent use in a serosurvey of sera collected in Italy, Japan, and the United States confirmed the distinct association of anti-delta antibodies (anti-HD) in patients with HBV infection (159, 164). Animal transmission studies clearly identified the delta agent as a separate virus that needed HBV to cause infection (158). These studies also characterized the clinical events of delta coinfections and superinfections. Over the next decade, considerable progress in the development of serologic tests for the diagnosis of acute delta infection and in the understanding of the molecular biology of this virus was made. In addition, knowledge about the clinical consequences of delta infection was expanded.

ANIMAL MODELS

Studies of transmission in chimpanzees (158) confirmed the potential pathogenicity and transmissibility of HDV. These studies were conducted shortly after the initial discovery by immunofluorescence of a new HDag-antibody system (157) and led to our understanding of the natural history of HDV infection. Sera obtained from two human carriers of HBsAg who expressed large amounts of intrahepatic HDAg in their livers were used as inocula in HBV-susceptible animals, in HBsAg chronic carriers, and in animals immune to HBV infection. Inoculation of the human sera into HBV-susceptible chimpanzees resulted in the development of HBV infection and the synthesis of HDV markers, effecting an HBV-HDV coinfection (Fig. 1). Inoculation of the sera into HBV carrier chimpanzees resulted in development of biochemical evidence of hepatitis; HDAg was concomitantly expressed in liver tissue and elicited an anti-HD response, a condition known as superinfection (Fig. 2).

HBV-HDV coinfections in experimentally infected chimpanzees can result in moderate to severe hepatitis characterized by a single or bimodal episode of elevated alanine aminotransferase (ALT) activity and the simultaneous expression of HDAg and HBcAg in hepatocytes. Both anti-HD and anti-HBc may appear simultaneously. An alternate course of expression of HBV and HDV markers of infection may also emerge. HBcAg may appear first, and this is followed by HDAg in infected hepatocytes; the reverse has also been observed, though less frequently. The order of expression is probably related to the titers of the infecting viruses. The appearance of HDAg, however, is not detected until after HBsAg begins to circulate as a marker of HBV infection. Coinfection often results in the transient existence of anti-HD, which is generally of relatively low titer and often of immunoglobulin M (IgM) anti-HD specificity (188).

Superinfections of chimpanzees, however, tend to be more severe, with shorter incubation periods than coinfections. Also, more HDAg is synthesized for longer times (Fig. 2). Chronic carriers of HBV superinfected with HDV usually develop an obvious course of hepatitis coincident with the synthesis of HDAg in the liver. Upon challenge with HDV, animals with circulating markers of HBV infection often demonstrate a diminution of both HBV DNA polymerase activity and syntheses of HBsAg, HBcAg, and HBV DNA. The molecular mechanisms effecting these changes are un-

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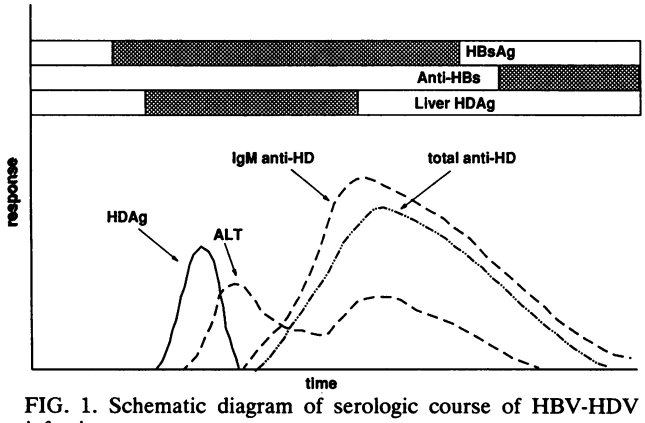

FIG. 1. Schematic diagram of serologic course of HBV-HDV coinfection.

known. In addition, superinfections result in more circulating anti-HD activity in the serum that appears to be lifelong, persisting after detectable HDAg synthesis in hepatocytes.

The chimpanzee model clearly demonstrated that HDAg was a marker for a transmissible pathogenic agent that requires the presence of HBV and that HDV interferes with HBV replication. In addition, the superinfection animal model has revealed that the HBsAg on the surface of the HDV virion is determined by the subtype that initiated the chronic carriage state and not by the subtype of HBV in the inoculum. In addition, successive passage of HDV in chimpanzees chronically infected with HBV produced a shortening of the incubation period before the appearance of HDAg and a progressive increase in the severity of the disease (152).

None of the initial superinfection experiments appeared to result in chronic HDV infection, as evidenced by the apparent disappearance of HDAg from the liver and plasma, which is the opposite to what occurs during superinfection in humans. In a study by Fields et al. (69), an HBsAg carrier chimpanzee superinfected with HDV demonstrated reappearance and persistence of HDAg in hepatocytes coinciding with a second and third abnormal elevation of ALT activity, indicating for the first time that a chronic HDV infection occurs in this animal model. Subsequently, Negro et al. (139), using a sensitive HDV RNA hybridization assay, reexamined stored sera from the initial superinfection experiments and found that more than 50% of the chimpanzees previously presumed to have developed acute, self-limited HDV infection had detectable signs of ongoing HDV replication for an average of 2.4 years after inoculation, while HDAg remained undetectable by immunofluorescence (IF) and immunoblot assays of both serum and liver. Thus, Negro et al. concluded that persistent HDV infection is frequent in superinfected chimpanzees, an observation consistent with the natural history of HDV superinfection in humans.

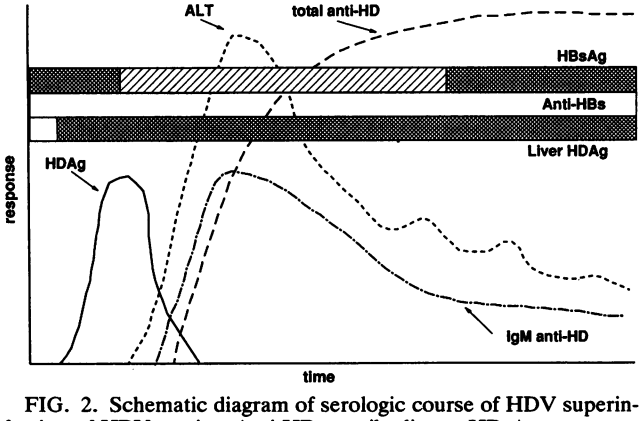

FIG. 2. Schematic diagram of serologic course of HDV superinfection of HBV carrier. Anti-HBs, antibodies to HBsAg.

Persistent HDV infection induced in a chimpanzee (69) was monitored for more than 3 years, and in collaboration with Govindarajan et al. (79), hepatocyte abnormalities similar to those observed in chimpanzees infected with hepatitis C virus (HCV) were observed by electron microscopy. These abnormalities included vacuoles, proliferated endoplasmic reticulum, and tubules. The tubular and reticular abnormalities were coincident with the reexpression of HDAg in liver biopsies and abnormal ALT levels. Canese et al. (26) reported identical ultrastructural abnormalities in chimpanzees infected with the same inocula.

In addition to ultrastructural changes, HDV and HCV have another feature in common. Shimizu et al. (182) developed from human lymphocytes a monoclonal antibody that immunoreacted with liver biopsy material obtained from both HDV- and HCV-infected animals (183). The monoclonal antibody detects the presence of microtubule aggregates that apparently are synthesized in response to HDV or HCV infection (98, 197).

The chimpanzee model provided conclusive evidence that the establishment of an HDV infection depends on an obligatory helper activity provided by HBV. Since the eastern woodchuck (*Marmota monax*) could also support HDV infection (146), this obligatory function could also be provided by the woodchuck hepatitis virus (WHV), another member of the hepadnavirus family with characteristics and tissue tropism similar to those of HBV. HDV of human origin from a second chimpanzee passage was inoculated into four woodchucks chronically infected with WHV. All four animals developed an HDV infection and showed serologic patterns similar to those observed previously in humans and chimpanzees. The HDAg found in woodchuck sera circulated as an internal component of a particle similar in size to the human HDV but was encapsulated by WHV surface antigen. This observation suggested that at least one obligatory function provided by either HBV or WHV was the availability of envelope proteins for HDV. HDAg from infected woodchucks, however, had biophysical properties identical to those of HDAg from infected chimpanzees. Similar to the chimpanzee model, successive passage in woodchucks resulted in greater expression of HDAg in the liver, suggesting successful adaptation of human HDV to the helper functions provided by WHV.

Subsequently, Ponzetto et al. (147) subpassaged HDV in woodchucks through five generations. Although HDV RNA became detectable in serum by sensitive hybridization techniques during the first passage, HDAg remained undetectable in serum until the fourth passage. With each successive passage, HDV RNA became detectable earlier, indicating a shortening of the incubation period from 6 weeks at first passage to 2 weeks at fifth passage. In four of five animals for which a 6-month follow-up was available, HDV RNA hybridization revealed persistent HDV infection that resembled chronic HDV infections in humans. Electron microscopic examination of HDV-infected hepatocytes revealed

ultrastructural changes similar to those found in liver tissue infected with either HCV or HDV.

Fields et al. (70) also demonstrated that woodchucks provided another animal model for studying the natural history of chronic HDV infection (for more than 500 days). The inoculum used for this woodchuck transmission experiment was obtained from a first-passage chimpanzee superinfection 400 days after inoculation, providing additional evidence of a persistent HDV viremia in the chimpanzee.

In addition to chimpanzees and woodchucks, the Pekin duck also supports hepadnavirus infections and would be a more convenient animal model to study HDV infection because of its smaller size. However, despite attempts in several laboratories, such studies have not been reproducibly successful (200).

Animal models will continue to contribute to our understanding of the pathophysiology and molecular biology of HDV. In addition, animal models will undoubtedly be used to determine whether a vaccine composed of recombinant expressed antigen affords protection to chronic carriers, who are the ones at most risk of developing severe liver disease due to HDV superinfection, and also to determine the effects of various antiviral treatment modalities on individuals who are already chronically infected.

METHODS OF DETECTING HDV

The primary laboratory tools for diagnosis of HDV infection are serologic tests for anti-HD. Tests for IgG anti-HD are commercially available in the United States (Abbott Laboratories, North Chicago, Ill.), while the other markers of infection, IgM anti-HD and HDAg, are widely available in Europe and can be imported into the United States for research use only. Tests for HDV RNA in serum, HDAg in serum (immunoblot), and HDAg in liver biopsies (IF or immunoperoxidase) remain tools of research facilities.

Detection of HDAg in liver tissue was first achieved by Rizzetto et al., who used a direct IF assay of biopsy material (157). Subsequently, human sera containing high titers of anti-HD and low titers of anti-HBc activity were labeled with horseradish peroxidase and used for the direct detection of HDAg by immunoperoxidase staining of frozen sections and paraffin-embedded sections from patients with chronic liver disease (26, 82, 194). Staining for HDAg in liver remains the gold standard for diagnosis with which all other detection methods are compared.

Rizzetto et al. reported that HDAg could also be detected in serum by using a RIA (164). More recently, enzyme immunoassays (EIA) for detecting serum HDAg have been developed (95, 179). In the assay described by Shattock and Morgan (179), Tween 20 is used to release the antigen from the HBsAg envelope. Because this assay employs a dissociating agent to disrupt HDAg–anti-HD immune complexes, it was more sensitive than earlier RIA formats and was able to detect circulating HDAg for a much longer time (179). Despite this improved HDAg detection, as the anti-HD titer continued to rise, the ability to disrupt the immune complexes that formed after release of HDAg from the HBsAg envelope resulted in the eventual inability to detect HDAg. This was especially true in chronic superinfection.

Although limited to the research setting, Western blot (immunoblot) analysis overcomes the primary limitation of serologic assays for HDAg. This method was applied independently by Bergmann and Gerin (12) and Bonino et al. (15) for the detection of HDAg in acute- and chronic-phase sera and in liver tissue from various human and animal sources. In the studies by Bergmann and Gerin (12), HDAg was purified from liver tissue by extraction with guanidine HCl and from serum specimens by pelleting through a 20% sucrose cushion. The electrophoretically separated HDAg-specific polypeptides were identified by Western blot analysis, using high-titered anti-HD serum and ^{125}I-labeled protein A. The results indicated that serum from acutely infected chimpanzees contained HDAg-specific polypeptides, which temporally correlated with the detection of HDAg by RIA. Unlike the RIA or EIA for the detection of serum HDAg, the immunoblot assay is unaffected by the presence of circulating anti-HD activity. The assay developed by Bonino et al. (15) differed primarily by using immunostaining with peroxidase-labeled anti-human IgG.

The first serologic immunoassay specific for anti-HD antibodies was developed by Rizzetto et al. (164). This competitive inhibition RIA utilized HDAg purified from the nuclei of human liver cells obtained at autopsy.

Subsequently, an EIA for the detection of anti-HD was developed by Crivelli et al. (53). The sensitivity of this assay was found to be intermediate between those of IF and RIA. In addition, a blocking EIA has been described by Fields et al. (69).

More recently, assays specific for IgM class anti-HD have been described. A capture RIA developed by Smedile et al. (188) is based on the selective binding of IgM from test sera to anti-human IgM (μ-chain specific) adsorbed to the solid phase. After the addition of the sera and the capture of human IgM onto the solid phase, HDAg extracted from the liver of an experimentally infected chimpanzee was added to interact with any IgM anti-HD captured by the solid phase. HDAg was detected by utilizing radiolabeled anti-HD, which could bind to the solid phase only when IgM anti-HD was present. Initially, this assay appeared to be very useful in detecting acute HDV infection and in serologically differentiating active HDV disease from nonpathogenic or past HDV infection. Dimitrakakis et al. (61) used this assay to distinguish between coinfection and superinfection by demonstrating that transient IgM anti-HD was observed only in patients who were coinfected with HBV and HDV, whereas in superinfections there was a prolonged elevation of IgM anti-HD activity. Farci et al. (64) extended these studies and demonstrated that IgM anti-HD activity persisted at high titers over many years in patients with unremitting or progressive liver disease but declined or disappeared in patients whose disease improved or resolved.

An EIA for IgM anti-HD was later developed by Shattock et al. (180). They suggested that, although the presence of HDAg is singly the best marker of HDV coinfection, IgM anti-HD is also useful when only late specimens are available.

Recently, the nature of IgM anti-HD has been examined by separation of 7S IgM monomers from 19S pentamers by fast protein liquid chromatography (208) and rate zonal centrifugation (125). Both studies conclude that expression of the 7S monomer appears to be an immunologic event specific for chronic HDV infections; thus, tests for 7S and 19S IgM anti-HD may be of value for differential diagnosis of acute and chronic infections.

Two recent studies have compared the sensitivities and specificities of various commercially available kits for the markers of HDV infection (13, 181). Both studies used sera from patients who were HBsAg positive. In one study (181), classification of patients with HDV infection was determined by in-house assays and documented seroconversion to IgM or total anti-HD. The greatest differences were obtained with

TABLE 1. Laboratory markers of HDV infection

Marker	Blood or tissue	Method	Detectability at given stage of illness[a]				
			Early acute	Acute	Convalescent[b]	Chronic	
						Symptomatic	No disease
HDAg	Serum	RIA, EIA[c]	+	±	−	−	−
		Immunoblot	++	+	−	+	?
	Liver	IF, IP[d]	++	++	−	++	−
Anti-HD							
IgM	Serum	RIA, EIA	±	++	±	++	−
IgG	Serum	RIA, EIA	±	++	±	+++	++
HDV RNA	Serum	cDNA or RNA hybridization	+	++	−	++	−

[a] +, detectable; −, not detectable; ±, may be detectable.
[b] In HDV-HBV coinfection.
[c] Must treat serum with detergent.
[d] IP, Immunoperoxidase.

serologic assays for HDAg, with sensitivities ranging from 100 to 24% (181). Anti-HDV assays had fewer discrepancies in sensitivities and specificities, with sensitivities ranging from 84 to 100% (13, 181).

Molecular cloning of the HDV RNA genome has permitted the application of an additional research tool for the diagnosis of HDV infection. Nucleic acid hybridization assays can detect the presence of HDV RNA in serum and in liver tissue; thus, as is true for HDAg assays, they can directly detect viremia. HDV RNA was first detected in serum by using a cDNA hybridization probe that represented only 8% of the total RNA genome (58, 190). The sensitivity of the assay was later improved significantly by using longer radiolabeled RNA probes (185) produced by in vitro transcription. Several investigators showed that persistence of HDV RNA in serum may indicate a progression to chronicity (25, 217). A method for detecting intrahepatic HDV RNA in frozen and formalin-fixed sections by in situ hybridization has also been developed (85); however, this assay does not appear to be more sensitive than the direct IF assay for HDAg.

Recently, the polymerase chain reaction has been applied to the detection of HDV RNA in both liver and serum samples (222). This method provides greater sensitivity than traditional hybridization techniques, but perhaps of greater importance is the application of this technique to the analysis of genetic variability of the HDV RNA genome.

The research diagnostic laboratory now has excellent assays available for the detection and monitoring of HDV infections. The immunoblot assay is available for the detection of HDAg in serum. In addition, the molecular hybridization assay is used for the detection of HDV RNA in serum (Table 1). Since these assays are noninvasive, they can be used in the research setting to monitor the course of HDV infections and the effects of various treatments without the necessity of obtaining a liver biopsy.

LABORATORY DIAGNOSIS OF CLINICAL DISEASE

Testing for markers of HDV infection should be considered when a patient shows clinical symptoms of acute or chronic hepatitis, particularly when the patient has fulminant hepatitis. In addition, when epidemiologic studies are done, testing for HBV carriers without overt disease should be considered. Tests for HDV RNA in serum, HDAg in serum (immunoblot), and HDAg in liver biopsies (IF or immunoperoxidase) remain tools of research facilities but could be useful in monitoring the clinical courses of patients with chronic HDV infection while they are under treatment.

Patients with HBV-HDV coinfection generally have only transient and low levels of anti-HD, including IgM anti-HD. In addition, although most patients with acute HBV-HDV coinfection will be HBsAg positive, a few will have cleared HBsAg by the time they come to a physician's attention. Therefore, patients with HBV-HDV coinfection should be positive for HBsAg, IgM anti-HBc, and anti-HD. In the rare circumstance in which a patient may have rapidly cleared HBsAg, IgM anti-HBc should be positive (Table 2). Since anti-HD may not be present until several weeks after the onset of illness, testing of sera in the acute and convalescent phases may be the best approach. The lack of licensed tests for HDAg and IgM anti-HD in the United States may result in underdiagnosis of HBV-HDV coinfections (5, 24, 61).

Patients with acute HDV superinfection will be HBsAg positive, IgM anti-HBc negative, and anti-HD positive. Patients with HBV-HDV superinfections generally have high levels of anti-HD. In patients with fulminant hepatitis, HDV markers in serum may not show up despite demonstrable HDAg in liver.

Chronic delta hepatitis is identified by the presence of HBsAg and very high titers (generally >1,000) of anti-HD. However, the diagnosis rests on finding HDAg in the liver or, in those patients with active viral replication, finding HDV RNA in serum (64, 188, 190).

PHYSICOCHEMICAL PROPERTIES OF THE VIRION

A mature HDV virion is a spherical particle with an average diameter of 35 nm (16, 93, 160) and a buoyant density of 1.25 g/cm^3 in CsCl (16, 160). An HDV particle contains a single-stranded, covalently closed RNA genome that is encapsulated with the only known HDV-encoded protein, the delta antigen, in a coat of surface antigen provided by the helper hepadnavirus, be it WHV or HBV. The helper hepadnavirus coat is composed of three forms of surface antigen referred to as pre-S1, pre-S2, and S polypeptides, which differ only in their amino termini. Infectious HBV particles (Dane particles) possess these proteins in a ratio of 1:1:4, respectively (94). In contrast, the ratio of these proteins in the HDV coat has been reported to be 1:5:95 (15);

TABLE 2. Clinical diagnosis of HDV infection

Clinical illness	Serologic results[a]						Interpretation
	HBV		HDV				
	HBsAg	IgM anti-HBc	HDAg	IgM anti-HD		Total anti-HD	
Acute hepatitis	+ or −	+	−	−		−	Acute HBV
	+ or −	+	+ or	+ or		+	HBV-HDV coinfection
	+	−	+ or	+ or		+	HDV superinfection
Chronic hepatitis	+	−	−	−		−	Chronic HBV
	+	−	−	+		+	Chronic HBV-HDV
None (asymptomatic)	+	−	−	+		+	Occult chronic HBV-HDV
	+	−	−	−		+	Chronic HBV with quiescent HDV

[a] +, detectable; −, not detectable; + or −, may be detectable.

thus, the coat of an HDV particle most closely resembles that of the hepadnavirus 22-nm noninfectious subviral particle normally found in large excess in serum. While Dane particles possess internal core structures that are released from the surface antigen coats after mild treatment with nonionic detergent, there are as yet no reports of a core or ribonucleoprotein structure (16, 160) for HDV; when HDV particles are treated with nonionic detergent, both genomic RNA and antigen are released, but they fail to show any association with one another.

STRUCTURE AND ORGANIZATION OF THE VIRAL GENOME

The structure of the HDV genome is unique among animal viruses. Its small, single-stranded RNA genome is approximately 1,700 nucleotides long and has an unusual secondary structure. Electrophoretic analysis of the HDV genome under native and denaturing conditions as well as electron microscopic studies (107, 210) revealed that HDV RNA is circular and, under physiologic conditions, assumes an unbranched rod-shaped form. These observations were consistent with the data obtained from cloning and sequencing the first HDV isolate. Computer analysis of the nucleotide sequence predicted that up to 70% of the bases were paired (210) to form an essentially double-stranded, rod-like structure (Fig. 3). While this genomic structure is unique among animal viruses, HDV bears a striking similarity to certain disease-causing agents of plants, namely, viroids, satellite RNAs, and satellite viruses (173). Like HDV, these pathogenic RNAs have small, single-stranded, circular RNA genomes that have rod-like secondary structures and, with the exception of viroids, require a helper virus.

The first HDV isolate to be sequenced was initially obtained from an Italian patient and had been serially passaged five times in chimpanzees prior to cloning (210). This isolate was also transmitted from chimpanzees to woodchucks and was subsequently cloned from infected woodchuck liver tissue (113). When the cloned HDV sequences were compared (108, 113), the lengths were the same (1,679 bases), and also extreme sequence conservation was found (up to 98.5% homology), indicating that little change occurred as a result of serial experimental transmission. The stability of these sequences contrasts markedly with that of certain human-derived HDV sequences. Complete genome sequences have been obtained from four virus isolates cloned directly from patient sera (40, 41, 127, 172), and partial sequences have been obtained from two Japanese isolates (104). The size of the genome varies from 1,676 nucleotides for a Taiwan isolate (41) to 1,683 nucleotides for an isolate obtained from the United States (127), and with the exception of one Japanese isolate, the sequences have 86 to 90% homology relative to the Italian isolate. Although only half of the genome of the Japanese isolate has been sequenced, it shares only 81% homology with the other isolates, making it the most divergent HDV isolate yet analyzed. Observed differences in the severity of outbreaks of delta hepatitis reported in different geographic areas (44, 89, 189) may be attributable to sequence variation of HDV. Although comparison of isolates obtained from such outbreaks may provide insight into which, if any, region(s) of the genome is

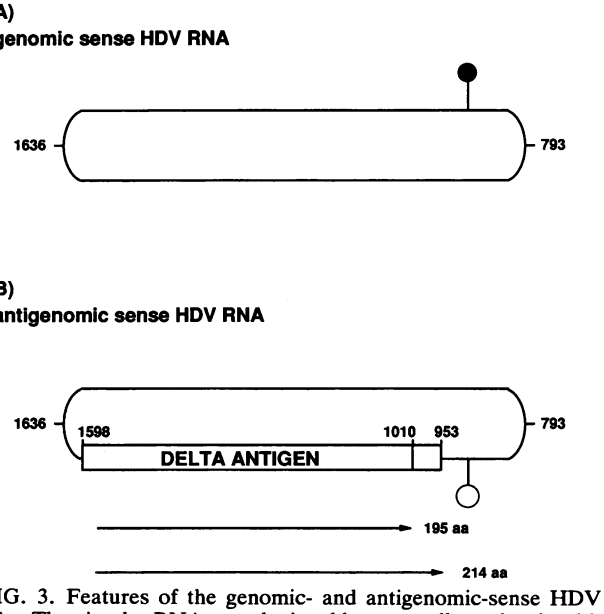

FIG. 3. Features of the genomic- and antigenomic-sense HDV RNAs. The circular RNAs are depicted here as collapsed rods with predicted ends at positions 793 and 1636. (A) Genomic-sense RNA contains a self-cleavage site (closed circle) between nucleotides 685 and 686. (B) The self-cleavage site on antigenomic-sense RNA is located between nucleotides 900 and 901 (open circle). The HDAg-coding region (open box) spans positions 1598 to 1010 for the small form of the HDAg and positions 1598 to 953 for the large form. aa, amino acids.

responsible for increased pathogenicity, the possibility that the apparent variability of pathogenicity is related to the nature of the associated HBV infection cannot be excluded.

VIRUS-ENCODED PROTEINS

Infected hepatocytes contain not only HDV genomic RNA but also a complementary strand that is referred to as antigenomic RNA (45). The antigenomic RNA is found in 10-fold-lower abundance in infected cells and presumably serves as a template for the synthesis of progeny genomic RNAs. Several open reading frames (ORF) capable of encoding more than 100 amino acids each were found on both RNAs (127, 211). However, to date, the only HDV-specific protein to be detected is the HDAg which is encoded by the largest ORF (ORF 5) of the antigenomic RNA (210).

Immunoblot analysis of proteins purified from both serum and liver has shown that two species of HDAg with estimated molecular weights of 24,000 and 27,000 react with sera from patients with chronic HDV infection (12, 15, 144). Weiner et al. (212) confirmed that both polypeptides are encoded by ORF 5. The two electrophoretic forms of HDAg are apparently a result of sequence microheterogeneity at nucleotide position 1012 of the HDV RNA. This microheterogeneity was first detected by Wang et al. (210, 211) during assembly of a composite sequence derived from seven overlapping clones derived from the serum of a single experimentally infected chimpanzee. Specifically, HDV genomic RNA sequences contain either cytosine or uridine residues at this position on the genomic RNA. The presence of a uridine residue results in the creation of an amber termination codon on the complementary antigenomic RNA. When this change occurs, protein translation terminates, resulting in synthesis of a 195-amino-acid form of HDAg. If, however, a cytosine residue is present, translation proceeds through to the next stop codon, resulting in synthesis of the larger, 214-amino-acid form of HDAg (Fig. 3). HDV cDNAs cloned directly from patient sera also have this specific ambiguity (40, 41, 218), and the phenomenon has been observed in vitro. Luo et al. (124) transfected cultured cells with an infectious HDV clone of known sequence and found that as much as 40% of the replicated HDV genome was specifically mutated at nucleotide position 1012, resulting in a change of the amber termination codon to tryptophan and thus altering HDAg from the small to the large form. This change was thought to occur via an "RNA duplex unwindase" activity that is present in most animal cells. The RNA unwindase activity deaminates adenosine, converting it to inosine, which results in replacement of adenosine with guanosine on HDV antigenomic RNA (9, 209). However, more recent studies by Casey et al. (33) and Zheng and Fu (221) prove that the editing occurs not on antigenomic RNA but on the genomic RNA. A uridine-to-cytidine change occurs on the genomic RNA, and this change is presumably transmitted to the antigenomic RNA by RNA-directed RNA synthesis.

HDAg is a highly basic protein that is phosphorylated at one or more serine residues (38). It has been shown to accumulate in the nuclei of infected hepatocytes (157) as well as in the nuclei of transfected cultured cells (38, 112, 126). It can act as an RNA-binding protein (38, 126), and the binding appears to be specific for both genomic and antigenomic RNA (121, 126). As discussed below, the small form of HDAg is essential for HDV genome replication, but its specific role has yet to be defined. The large form, in contrast, has been proven to be essential for virus assembly (37, 170).

VIRAL REPLICATION

Studies of HDV transfection have helped delineate the role of the helper hepadnavirus. Although it is possible that HBV has additional functions in vivo, the results of several studies suggest that its role is limited to packaging of HDV particles.

The HDV genome can replicate in nonliver cells and in the absence of HBV helper. Using a trimer of the complete HDV sequence, Kuo et al. (112) transfected monkey kidney (COS-7) and human hepatocellular carcinoma (HuH7) cell lines. The recombinant plasmid containing the trimer was constructed such that, following transfection, genomic HDV RNA was synthesized under control of the simian virus 40 late promoter. Nine days after transfection, in addition to the expected genomic-sense RNA, a significant amount of HDV antigenomic RNA was detected, indicating that viral replication had occurred. The electrophoretic mobility of this antigenomic RNA was identical to that of RNA in the liver of an HDV-infected chimpanzee.

The requirement of HDAg for genome replication was also demonstrated by Kuo et al. (112). When they performed transfections with a trimer containing a frameshift mutation within the HDAg-coding region, genome replication was reduced 40-fold. This reduction, however, could be alleviated if the cells containing the mutant genome were cotransfected with a plasmid expressing a correct copy of HDAg. The requirement of HDAg for genome replication was also reported by Glenn et al. (74), who transfected cultured fibroblasts with monomers of HDV RNA. Evidence of genome replication could be detected only in those cells that had first been transfected with a construct that directed the synthesis of HDAg, confirming the requirement of this protein for efficient replication.

Several laboratories have reported not only that HDV can replicate but also that it can be packaged in vitro when the helper hepadnavirus coat proteins are provided. Following cotransfection of HuH7 with two plasmids, one containing a trimer of HDV and the other containing the complete HBV genome, Wu et al. (216) demonstrated that both viruses were packaged and released into the culture medium. Physicochemical analysis of the HDV particles indicated that they were identical to virions found in infected serum. As predicted from animal transmission studies, Ryu et al. (170) showed that HDV can be packaged in vitro by using surface antigens from either HBV or WHV. Using various combinations of recombinant DNAs, they demonstrated that provision of just the S polypeptide was sufficient to package the HDV genome, although infectivities of these particles lacking the pre-S1 and pre-S2 regions of the coat protein were not determined.

Recently, the possible biological significance of the alternate forms of HDAg has been investigated. Chao et al. (39) examined the abilities of the small (195-amino-acid) and large (214-amino-acid) forms of HDAg to support HDV replication in transfected cells. Cultured cells were cotransfected with a plasmid expressing either the small or the large form of HDAg along with a multimer of HDV incapable of synthesizing a functional HDAg. While the HDV genome was able to replicate in cells expressing the small form of HDAg, no evidence for replication was found in those cells expressing only the large form. Indeed, when both forms of HDAg were present in the cell, the large form exhibited a potent inhibi-

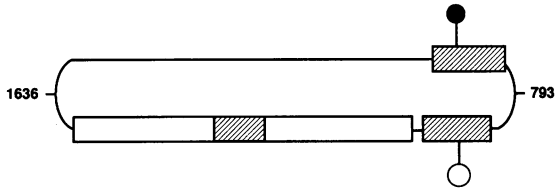

FIG. 4. Conserved regions of the HDV genome. The open box indicates the HDAg-coding region on the antigenomic RNA. Closed and open circles show the positions of the self-cleavage sites on genomic and antigenomic RNAs, respectively. The self-cleavage sites are located within two conserved domains (hatched boxes) that span nucleotides 656 to 769 and 844 to 963. A third conserved region (nucleotides 1264 to 1344) is located within the middle one-third domain of the HDAg-coding region.

tory effect; thus, the large form appears to be a dominant negative inhibitor (97).

Similar results were obtained by Glenn and White (75). They speculated that the specific sequence alteration that occurs at nucleotide position 1012 during HDV genome replication and that leads to synthesis of the large form of HDAg is an essential part of the HDV life cycle. This switch not only represents a mechanism for repressing further genome replication but also appears to be necessary for genome packaging. Chang et al. (37) and Ryu et al. (170) found that the large form, but not the small form, of HDAg is required for assembly of HDV particles in transfected cells. The ability of the large form to repress replication may explain various clinical outcomes of HDV infection. If the switch to synthesis of the large form does not occur in time, acute infection may rapidly proceed to fulminant hepatitis.

Several lines of evidence suggest that HDV, which is structurally similar to plant viroids, may share with them a similar replication strategy. It has been proposed that the plant pathogens replicate by RNA-directed RNA synthesis via a rolling-circle model (19). DNA replication intermediates are not utilized in the replication strategy; rather, multimeric RNAs are synthesized by using cRNA as a template for genome replication. Consistent with this replication model is the fact that while HDV DNA intermediates have not been found in infected livers, multimeric RNAs of both genomic and antigenomic sense have been reported (45). In viroids, the multimers are processed to monomeric circular molecules by site-specific cleavage and subsequent ligation (72, 73, 103, 151). Surprisingly, these activities occur in the absence of protein and thus resemble the self-splicing of introns (35), i.e., ribozyme activity. Self-cleavage of HDV genomic (114) and antigenomic (114, 177) RNAs occurs in vitro. The reverse reaction, self-ligation, has also been observed (178, 215). As predicted, these reactions are site specific and occur between nucleotide positions 900 and 901 on antigenomic RNA and positions 685 and 686 on genomic RNA (Fig. 3).

Although, as discussed above, HDV isolates show considerable sequence variation, it has become clear that the HDV genome contains discrete conserved regions that correspond to functionally important regions of HDV RNA (18, 40, 41). The self-cleavage sites for genomic and antigenomic RNAs fall within two of these highly conserved regions; a third region is located within the HDAg-coding sequence (Fig. 4). The sequence conservation of replication-important areas has prompted Branch et al. (18) to propose a two-domain model for HDV RNA. The collapsed rod-like structure of HDV RNA is divided into two parts. Elements required for replication are clustered at one end of the molecule into what they refer to as a viroid-like domain. Of the remaining three-quarters of the genome, almost half is dedicated to HDAg, while the remainder may stabilize the molecule. The authors speculate that HDV may have originated by the association of an mRNA and a free-living RNA having properties similar to those of the plant pathogens discussed above.

EPIDEMIOLOGY

Given the dependent characteristics of HDV, the epidemiology of HDV infection closely parallels that of HBV. Worldwide, there are approximately 300 million HBsAg carriers, and available data indicate that no fewer than 5% of these are infected with HDV (162). The reservoirs of infection and the transmission patterns are similar to those of HBV. However, there are differences in the global distributions and efficiencies of transmission of HBV and HDV.

Studies of the seroprevalence of HDV infection should be interpreted with caution, since many studies involve small or select populations. The seroprevalence of HDV infection in patients with acute, chronic, or fulminant hepatitis may be 3 to 10 times higher than that in asymptomatic HBV carriers, because HDV infection enhances the severity of acute and chronic hepatitis (90). Consequently, it is most instructive to evaluate the relative frequencies of HDV infection in asymptomatic HBV carriers and in persons with chronic hepatitis B and compare these with frequencies in the same segments of other populations (90).

Relative to HBV endemicity in the population, Hadler and Fields (90) defined four broad classifications of HDV infection: (i) very low endemicity when HDV prevalence is 0 to 2% in asymptomatic HBV carriers and less than 10% in patients with chronic hepatitis B; (ii) low endemicity when HDV prevalence is 3 to 9% in asymptomatic HBV carriers and 10 to 25% in patients with chronic hepatitis B or cirrhosis; (iii) moderate endemicity when prevalence is 10 to 19% in asymptomatic HBV carriers and 30 to 60% in patients with chronic hepatitis B; and (iv) high endemicity when prevalence is above 20% in asymptomatic carriers and above 60% in patients with chronic hepatitis B. The worldwide distribution of HDV infection is shown in Fig. 5 and is compared in Fig. 6 with the distribution of HBV infection.

In general, two patterns of transmission have been described. In areas of high endemicity such as southern Italy, Africa, and South America, transmission is thought to occur through person-to-person contact, whereas in areas of low endemicity such as the United States transmission is limited to groups with frequent percutaneous exposure to blood, such as intravenous drug users and hemophiliacs (161). There are, however, varying patterns in the worldwide epidemiology of HDV infection.

In regions of the world with high HBV endemicity, HDV prevalence varies widely. In Africa, HDV prevalence is high in northern Kenya (86), the Central African Republic (54, 116), and rural Niger (193); moderate in Nigeria (4), Djibouti (1), Somalia (2), northern Uganda (57), and central Burundi (57); and low in Ethiopia (154), Liberia (76), and South Africa (62). Several studies have shown that seroprevalence may vary among different groups in the same country. In Ethiopia, anti-HD seroprevalence is approximately 17% in the Tigre ethnic group but only 2% in the Oromo group (154). In addition, in asymptomatic HBV carriers, anti-HD sero-

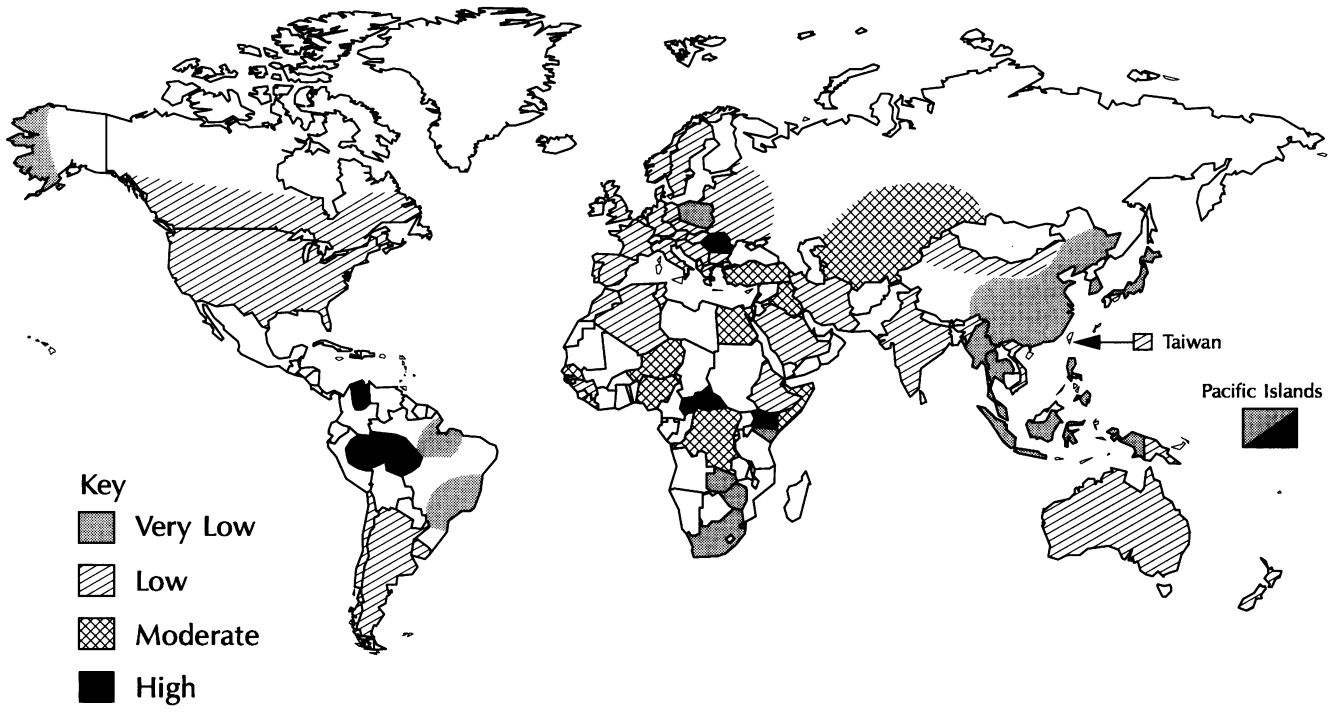

FIG. 5. Prevalence of HDV infection worldwide.

prevalence in Kenya ranges from 1% in the south to 31% in the north (86).

By contrast, throughout China, Japan, and Southeast Asia, anti-HD seroprevalence is very low except for several isolated groups (43, 44, 50, 102, 134, 162, 163, 196, 206). High levels of HDV infection have been found in intravenous drug users in Taiwan (84%) (43, 49) and in approximately 20% of Taiwanase prostitutes (87). In contrast to studies showing low prevalence of HDV infection in Southeast Asia and China, studies of over 2,300 HBsAg-positive subjects from six countries and nine islands in the Western Pacific revealed foci of high anti-HD seroprevalence in the islands of Western Samoa, Niue, Nauru (60), and the Pacific Republic of Kiribati (202). Data from American Samoa indicated that 11% of HBsAg carriers were HDV seropositive. The age-specific rates were low in children, in young adults under the age of 30 years (<5%), and in adults older than 40 years (20 to 33%). The disease appeared to be benign clinically, with only 5% of patients giving a history of clinical hepatitis and only 7% having abnormal levels of transaminases (87). Countries with low anti-HD seroprevalence include the Cook Islands, Tahiti, New Caledonia, Vanuatau, New Zealand, and Fiji (60, 153). The high prevalence of HDV infection in some isolated Pacific island populations suggests that once HDV infection is introduced into a population, it spreads rapidly. Unexplained behavioral and/or racial factors may influence the penetration of HDV into other Oriental populations (90).

South America, which has areas of high and low HBV prevalence, also has widely varying HDV prevalence. In the south, where HBV prevalence is low, HDV prevalence is very low. Studies in Uruguay, Chile, Argentina, and the southern portions of Brazil have shown HDV prevalence rates of less than 2% in HBsAg carriers (32, 68). In the northern part of South America, HDV prevalence varies widely. In Lima and select Andean regions of Peru (Huanta, Ayacucho); Medellin, Colombia; the eastern Amazon; and Sao Paulo, Brazil, the HDV prevalence is very low, whereas in the Amazon basin, among certain Indian groups in Venezuela, and in the Santa Marta region of Colombia, the prevalence of infection is among the highest in the world (11, 32, 71, 89, 123, 184, 195).

From 1979 to 1982, 149 Yucpa Indians living in the Perija mountains of western Venezuela developed hepatitis with an unusually high mortality rate. Serologic data from an investigation of this outbreak indicated that most infections were secondary to HDV superinfection of hepatitis B carriers and that more than 60% of infections progressed to chronic liver disease (89). Subsequent to this investigation, two historic entities of fulminant hepatitis, Labrea hepatitis occurring in the Amazon basin and hepatitis of the Sierra Nevada de Santa Marta occurring in northern Colombia, were found to be associated with HDV infection (11, 123). Cases from these regions demonstrated similar and unique histopathologies consisting of microvesicular fatty infiltration and eosinophilic necrosis of hepatocytes (11, 149). Studies in the Boca do Acre region of the southern Amazon Basin evaluating the role of HDV in the cause of Labrea hepatitis show several characteristic features of the high HDV endemicity in the central and western Amazon Basin. HDV infection was found in 24% of asymptomatic HBsAg carriers, 29% of patients with acute hepatitis B, 74% of patients with fulminant hepatitis B, and 100% of patients with chronic hepatitis B. This high HDV prevalence overall varied widely for different ages and geographic regions. Prevalence of HDV antibody was <10% in children younger than 10 years of age, 26% in children aged 10 to 14 years, and >40% in persons older than 14 years. HDV prevalence also varied by village, ranging from 0 to 60% (11). Recently, an outbreak of severe delta hepatitis in the Yanomami communities of the Upper Orinoco basin in southern Venezuela has been described (203).

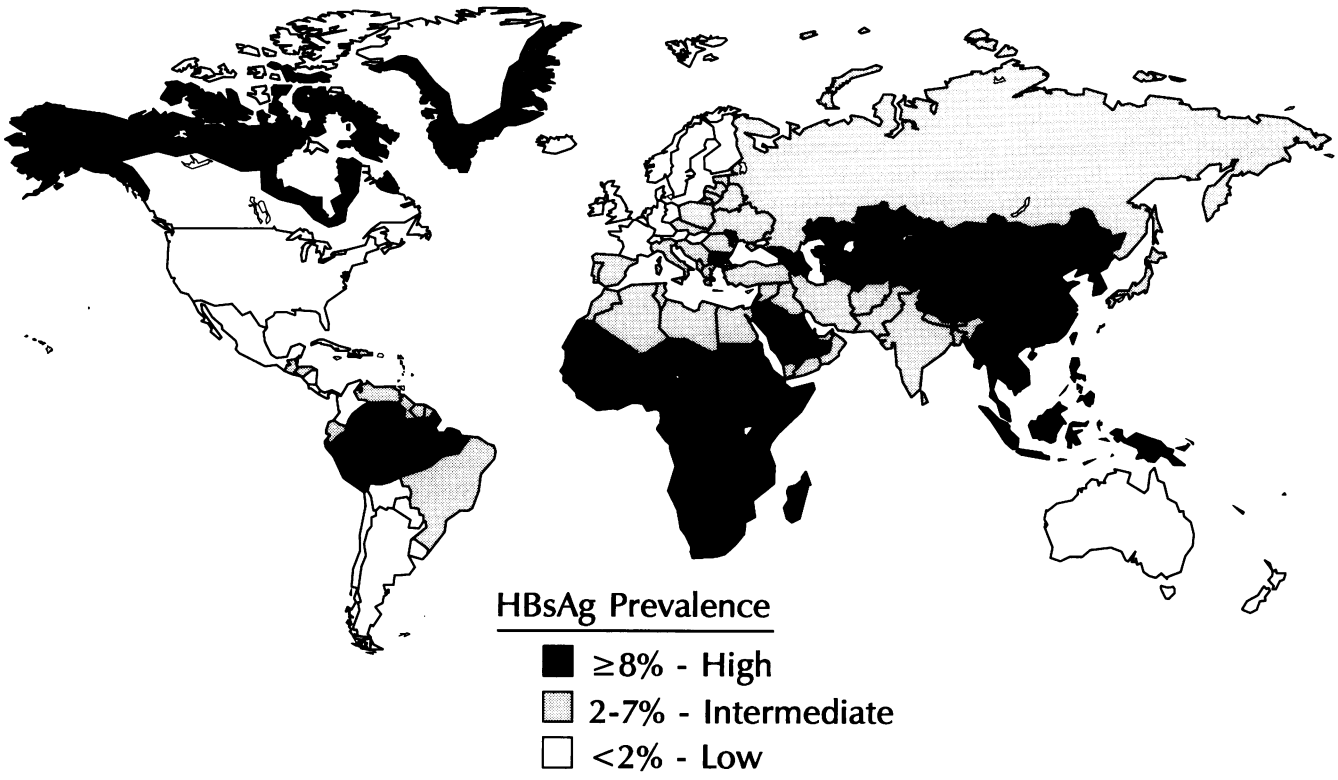

FIG. 6. Geographic distribution of chronic HBV infection.

Disease transmission has been extensively evaluated in long-term follow-up studies of HBV carriers in the Yucpa population. Follow-up of 216 carriers showed an initial HDV seroprevalence of 34%. Over the next 5 years, 36 new infections were identified, for an annual attack rate of 10% (87). New infections were most common in children (1 to 9 years) and young adults (15 to 19 years). The most significant risk factor for acquisition of disease identified in this cohort study was living in a household with someone who had an acute case of HDV infection. The data from these investigations indicate that HDV is spread most readily in households that include an HBV carrier. In addition, the age-specific patterns of illness suggest that disease is spread by sexual transmission in young adults and contact through open skin lesions in children (present in >40%) (87).

The prevalence of HDV infection in areas of the world with low or moderate HBV endemicity is also low to moderate. HDV prevalence rates of 0 to 19% occur in HBsAg carriers from Belgium, Germany, Hungary, Poland, Spain, Portugal, Yugoslavia, Norway, Jordan, Saudia Arabia, Yemen, Lebanon, Iran, and North India (23, 156, 162, 176, 204). In some countries, such as Italy, HDV endemicity is variable. HDV appears to be endemic in the general population of southern Italy. By contrast, HDV infection in northern Italy predominates among southern emigrants in industrial towns and parenteral drug users (189).

In the United States, the prevalence of HDV infection in the general population is low. The prevalence of HDV infection in HBsAg-positive volunteer blood donors varies from a low of 1.4% in one southeastern region of the country to 12.4% in southern California (129, 137). The prevalence of HDV infection in various high-risk groups is shown in Table 3. The two groups with the greatest risk of HDV infection include intravenous drug users and hemophiliacs (55, 56, 115, 129). Homosexual men, persons in institutions for the developmentally disabled, hemodialysis patients, and groups with high rates of HBsAg carriage generally have been found to have low rates of HDV infection (96, 129, 155, 192, 213). The prevalence of HDV infection is low among Southeast Asian refugee immigrants and essentially absent among high-risk populations, including North American and Canadian Eskimos and other Asian refugees (129).

TABLE 3. Prevalence of HDV infection among HBsAg carriers in certain groups in North America

Group	% Prevalence	
	HBsAg	Anti-HD[a]
Blood donors	0.1–0.5	1.4–8.0
Parenteral drug abusers		
With AIDS	15.1	0
Without AIDS	5.1	21.4
Hemophiliacs	3–5	48–80
Homosexual men	5–10	15
Hemodialysis patients	2–5	8–20
Mentally retarded	5–15	0–4, 30
Southeast Asian refugees	5–15	2.4–8
Other Asians	2–10	0
Alaskan Eskimos	5–15	0.6
Canadian (Inuit) Eskimos	5–15	0
American Samoa inhabitants[b]	8	9
Marshall Islands inhabitants[b]	5–10	0

[a] Modified from Maynard et al. (129).
[b] U.S. jurisdictions in the Pacific.

Although HDV is generally an endemically transmitted disease, outbreaks have been recognized. Outbreaks of HBV-HDV coinfection have been reported most frequently among intravenous drug users. A study conducted in Sweden from 1970 to 1980 indicated that once HDV is introduced it may spread rapidly through a high-risk, susceptible population. Prior to 1975, no simultaneous infections with HBV and HDV were found. However, after introduction of HDV in 1973, between 18 and 44% of intravenous drug users with acute hepatitis B were also infected with HDV (92). A similar outbreak occurred among parenteral drug users in Worcester, Mass., from 1983 through 1985. Among drug users with acute hepatitis B, 54% also had HDV infection (118). Outbreaks have also been reported from Italy (29). An unusual outbreak of HBV-HDV coinfection in butcher shop employees was thought to be secondary to contamination of open skin lesions obtained during work (132).

Outbreaks of HDV superinfection among HBsAg carriers have been reported from South America and Africa (11, 54, 89, 123). The outbreak among the Yucpa Indians of Venezuela represents the first such outbreak described. Transmission has generally occurred among HBV carrier children and young adults and is thought to be secondary to bloodborne transmission through open skin lesions. Outbreaks of HDV superinfection of HBV carriers, however, are not limited to this part of the world. A recent outbreak in the foothills of the Himalayas in South Kashmir, India, suggested that overcrowding and person-to-person contact were the most likely mechanisms of transmission, and the attack rate was highest among young adults (106). In the United States, outbreaks of HDV superinfection are uncommon. However, a 1985 study of HDV infection in facilities for the developmentally disabled in Illinois detected anti-HD in 71 (30%) of 238 of the hepatitis carriers. A case control study found that infection most likely occurred in one large facility during the 1950s and 1960s. Because of overcrowding and understaffing during this period, transmission could have occurred through inapparent percutaneous exposure to infective blood or through human bites, sexual contact, or shared objects. In the last 20 years, no transmission has been documented in these institutions for the developmentally disabled because policy changes have improved living standards in these facilities (96).

Although the worldwide distribution of HDV has been extensively studied, differences in seroprevalence, disease transmission, and disease severity have not been well explained. The severity of acute disease and the progression to chronic liver disease among patients with HDV infection in the Amazon Basin contrast sharply with the same features of disease in areas such as American Samoa, where the disease appears to be clinically benign. Although this distinction remains unexplained, the possibility of HDV strain differences and/or nutritional and genetic cofactors needs to be further explored.

Transmission of HDV is restricted to those mechanisms described for HBV transmission and include direct percutaneous exposure to contaminated blood through parenteral use of drugs or through a blood product transfusion, perinatal transmission from mother to child, sexual contact, and inapparent transmission through open skin lesions or environmental contamination.

Parenteral transmission among intravenous drug users has been well documented from around the world. Seroprevalence rates have ranged from 25 to 91% in asymptomatic HBsAg-positive carriers (8, 34, 59, 101, 109, 128, 130, 142, 207). In one study, anti-HD was associated with greater age, greater duration of intravenous drug use, and presence of chronic liver disease (142). In a study evaluating the prevalence of HDV markers in parenteral drug users with and without AIDS, significant differences were found in the prevalences of HDAg, anti-HD, and HBsAg. Patients with AIDS were more likely to be positive for HDAg (5.7% versus 0.8%; $P < 0.05$) and HBsAg (15.1% versus 5.1%; $P < 0.05$). However, patients with AIDS were less likely to be anti-HD positive (0% versus 21.4%; $P < 0.01$), suggesting that the immunosuppression associated with AIDS may allow for either persistence or reactivation of both HDAg and HBsAg (109).

Hemophiliacs who receive clotting factors are also at increased risk of acquiring HDV infection. Rizzetto et al. found a 53% overall prevalence of anti-HD in HBsAg-positive hemophiliacs from Italy, Germany, and the United States (163). Rosina et al. reported an anti-HD prevalence of 34% in HBsAg-positive hemophiliacs from western Europe and the United States who had received multiple transfusions from 1973 to 1983 (169). However, there was no evidence of HDV infection in HBsAg-positive hemophiliacs from Brazil, the former German Democratic Republic, and Australia who had received single or minipool volunteer plasma (169). Similar results have been found in multiply transfused patients with thalassemia and sickle cell disease from Saudi Arabia, with anti-HD seroprevalence rates ranging from 0 to 27% (63). Seroprevalence rates among hemophiliacs in the United States seem to have remained stable. Studies conducted during 1987 and 1988 have shown an anti-HD prevalence of approximately 35% among multiply transfused HBsAg-positive hemophiliacs (10, 205). Screening for HBsAg provides a high degree of safety in preventing infection with both HBV and HDV; however, HBsAg-negative blood still may rarely contain infectious HBV and thus be a vehicle for transmission of HDV. Although the risk of acquisition of either infection is low in the general population, the risk of acquiring an HDV infection is substantially greater for HBsAg carriers, particularly hemophiliacs who require multiple transfusions. Consequently, it has been recommended that HBsAg carriers be given only blood derivatives obtained from single or minipool volunteer donors (169).

Inapparent transmission through open skin lesions has been postulated among the Yucpa Indians, in whom transmission of HDV was associated with high frequencies of impetigo, scabies, insect bites, and dermatoses (89). Environmental contamination has been the suspected mechanism of transmission in a nosocomial outbreak associated with hemodialysis units (117). In settings for the developmentally disabled in which HDV transmission has been recognized, the route of transmission is presumably related to handling of shared objects (i.e., toothbrushes), human bites, or exposure of open skin lesions to infective material in the environment.

Sexual transmission has been documented for several outbreaks in which a relatively high frequency of HDV infection was found among contacts of intravenous drug users (29, 118, 133). In addition, spouses of HDV carriers have high rates of HDV infection (14). In Taiwan, 23% of HBsAg-positive licensed prostitutes who seldom engaged in intravenous drug use were anti-HD positive (42). A prospective study also conducted in Taiwan during 1989 identified 203 acute hepatitis B cases. Of these, 17% (34 people [30 men, 4 women]) also had serologic evidence of acute HDV infection. Of the 30 HDV-positive men, 27 (90%) had had sexual intercourse with prostitutes in the 3 months prior to

their illness and no patient had other risk factors for the acquisition of HDV infection (119). Studies among homosexual men who do not use drugs have indicated various rates of HDV infection, and rates have ranged from 0 to 40% in HBsAg-positive patients (131, 145, 192, 213). In the United States, the prevalence of anti-HD in HBsAg-positive homosexual men was 15% in Los Angeles, 9% in San Francisco, 1% in Pittsburgh, and 0% in Chicago. Multiple regression analysis of risk factors indicated that HDV infection was independently associated with intravenous drug use, number of sexual partners, and rectal trauma (192). At least one study has indicated that the majority of HDV infections in homosexual men resulted from superinfections of HBV carriers; however, this has not been a consistent finding (55). Sixty to 70% of homosexual men have markers of HBV infection (3), but with rare exceptions, fewer than 15% have markers of HDV infection, suggesting that sexual transmission of HDV is less efficient than sexual transmission of HBV.

Although few studies have been conducted, perinatal transmission does not appear to be of great importance. Zanetti et al. (219) found that vertical transmission of HDV was dependent on the hepatitis B e antigen (HBeAg) status of the mother. Of 6,111 pregnant women from Northern Italy who were evaluated, 164 (3%) were HBsAg positive, and of these, 9 (5%) were HBeAg positive. Seven (4%), one with HBeAg and six with anti-HBeAg antibodies (anti-HBe), were positive for anti-HD. Of the babies born to these seven women, only one, the baby born to the mother who was HBeAg positive, developed elevated levels of anti-HD (219).

Body fluids such as semen or saliva have not been evaluated for the presence of HDAg. It is likely that semen and vaginal secretions contain infectious material, since sexual transmission has been well documented in several studies. There is no evidence to suggest that other routes of transmission known to occur with hepatitis A (fecal-oral) or hepatitis E (waterborne) or that airborne transmission occurs with HDV.

CLINICAL FEATURES

As indicated above, infection with HDV occurs as a simultaneous coinfection with HBV or as a superinfection in an HBsAg carrier. Although the acute clinical presentations of a coinfection and a superinfection are indistinguishable, the long-term sequelae differ markedly. Chimpanzee inoculation studies have indicated that simultaneous infection with HBV and HDV results in an incubation period that ranges from 6 weeks to 6 months and that may be dependent on the HBV infectivity of the inoculum (158).

The symptom complex associated with acute HDV infection is similar to other causes of acute viral hepatitis; however, a biphasic course of illness is more likely in HBV-HDV coinfections than in acute infections with HBV alone. Of 57 patients in Sweden simultaneously infected with HBV and HDV, 8 (14%) had a biphasic illness characterized by elevations in bilirubin and aminotransferases. Only 2 (3.5%) of the 57 patients became HBsAg carriers (135). Similarly, Caredda et al. found that 32% of patients (68 of 216) coinfected with HBV and HDV had a biphasic illness, with a 2- to 5-week interval between phases, compared with 16% of patients (26 of 162) with acute infection with HBV alone (28, 30).

Coinfection with HBV and HDV results in fulminant hepatitis significantly more often than does infection with HBV alone. This association was first suggested by Smedile et al. (191), who detected HDV serum markers in 19% of patients (101 of 532) with acute benign hepatitis B compared with 39% of patients (43 of 111) with fulminant hepatitis B (191). Similarly, Govindarajan et al. (77) found that the prevalence of serologic HDV markers was 29% in patients with fulminant hepatitis B compared with 4% in patients with benign acute hepatitis B. Other studies have shown the prevalence of HDV markers in patients with acute fulminant hepatitis B to be as high as 50% or more (111, 199). Worldwide, the percentage of cases of fulminant hepatitis B that can be attributed to coinfection with HDV ranges from 3 to 25%. Taiwan and India have the lowest rates (3 and 6%, respectively), while the United States and Italy have the highest rates (24 and 25%, respectively) (174). Mortality rates for patients with fulminant hepatitis secondary to coinfection with HBV and HDV have varied considerably. Several studies have indicated a lower mortality with coinfections than with fulminant hepatitis B alone (77, 174). However, when age-specific mortality rates were analyzed, no significant differences were noted (77).

Most studies of HBV-HDV coinfections have indicated that the majority of patients do not develop an HBsAg carrier state. Of 57 patients identified in Sweden who had HBV-HDV coinfections, only 2 (3.5%) developed a chronic HBsAg carrier state compared with 25 (4.7%) of 535 patients with hepatitis B infection alone (135). Caredda et al. (28) found that 2.4% of patients with coinfections and 1.2% of patients with acute hepatitis B became HBsAg carriers. However, all five patients (males aged 18 to 25 years) with HBV-HDV coinfections who became HBV carriers had a rapid progression to chronic liver disease, which advanced to cirrhosis in three patients within several months.

In 16 to 86% of patients, HDAg can be detected in the serum approximately 1 to 10 days after the onset of clinical illness (5, 61, 135), but titers decline fairly rapidly and are rarely detected more than 21 days after the onset of illness (61). In an Australian study, IgM antibodies were detected within 2 to 3 weeks after the onset of illness, with a mean duration of 22 days, and generally were not detected more than 2 to 3 months after the onset of illness (61). In addition, blocking anti-HD was detected in only 50% of patients with detectable IgM antibodies. Two distinct serological patterns were observed in patients coinfected with HBV and HDV: (i) a transient IgM response, with development of long-lasting blocking anti-HD; and (ii) a transient IgM response, with failure to develop anti-HD (61).

Several studies have suggested that HDV may suppress HBV replication during acute coinfection with HBV and HDV. Morante found that the levels of HBV DNA were significantly lower in patients coinfected with both viruses than in patients infected with HBV alone (136). Other investigators have found that during acute coinfection there is significant suppression of HBsAg synthesis, which leads to the clearance of HBsAg prior to the clinical manifestations of HDV infection (27). The diagnosis of acute coinfection with HBV and HDV in situations in which HBsAg is not present is dependent on the detection of IgM anti-HBc and IgM anti-HD. In one study of patients with fulminant hepatitis B and rapid clearance of HBsAg, 50% were positive for anti-HD IgM, confirming previous studies documenting the role of HDV suppression of HBV replication (199).

Superinfection of an HBsAg carrier with HDV produces acute hepatitis in approximately 50 to 70% of patients (17, 51, 135). Asymptomatic infection can occur, as evidenced by the detection of HDV antibodies in 5% of HBsAg carriers without a prior history of hepatitis (6). Biphasic illness,

which can be seen in patients coinfected with both HDV and HBV, is not often seen in HBsAg carriers superinfected with HDV (28, 135). HDV infection should be considered in the differential diagnosis of acute hepatitis in an HBsAg carrier. An interesting study from Taiwan, an area with a low prevalence of HDV infection, suggested that the etiology of acute hepatitis in asymptomatic HBsAg-HBeAg-positive carriers was often related to immune clearance of HBV in the natural history of a chronic HBV infection. However, in patients who were HBsAg and anti-HBe positive, 55% of the acute episodes of hepatitis were related to HDV superinfection (48).

Animal transmission studies have shown that the inoculation of HBsAg carrier animals enhances and accelerates the synthesis of HDAg. The incubation period is shorter than that for HBV-HDV coinfection and ranges from 2 to 8 weeks (158). Presumably, the established presence of HBsAg allows the relatively rapid synthesis of HDAg. In chimpanzee inoculation studies, the incubation period has been dependent on the titer of the HDV inocula; however, severity and duration of illness were independent of HDV inocula (148).

Similar to the situation with HBV-HDV coinfection, fulminant hepatitis occurring as a result of superinfection of HBsAg carriers is more common than in acute HBV infections. In a worldwide study of fulminant hepatitis, 14% of cases (54 of 377) were a result of an HDV superinfection (174). Similarly, in an Italian study, 42% of the fulminant hepatitis cases (18 of 43) associated with HDV could be attributed to a superinfection (191). Fulminant hepatitis of HBsAg carriers has been well documented in outbreaks in South America (11, 20, 149).

The single most important feature differentiating HBV-HDV coinfection from HBV-HDV superinfection is the development of chronic HDV carriage in those HBV carriers who become superinfected, with the possibility of progression to chronic active hepatitis or cirrhosis (55, 66, 187). Most studies have indicated that HBV carriers superinfected with HDV generally develop and maintain serologic markers that indicate ongoing viral replication, including high titers of anti-HD and persistence of IgM anti-HD, serum HDV RNA, and intrahepatic HDAg (22, 64, 187, 188, 190). However, this is not always the case, and patients may lose detectable HBsAg secondary to HDV suppression of HBV replication (46, 91, 110, 135). Several investigators have also reported that in some instances HBsAg carriers superinfected with HDV and examined 10 to 49 months after the acute episode became negative for total anti-HD, suggesting that HDV superinfection may be a self-limited disease with clearance of both HBV and HDV infections (47, 198).

The natural history of progression of disease has been investigated in numerous studies, with conflicting results. In uncontrolled studies, several investigators have documented progression to cirrhosis in 20 to 60% of patients with markers of HDV infection within 2 to 6 years (78, 165, 175). In controlled studies, patients with markers of HDV infection were more likely to have rapid progression to cirrhosis than patients without markers of HDV infection (67, 171, 217). This has also been shown in recent studies that used the polymerase chain reaction (186). However, this finding has not been a consistent one, and several investigators have not documented rapid progression to cirrhosis in those patients with HDV markers (51, 120). The outcome of HDV infection, at least in one study, was unrelated to the severity of the initial morphologic lesion observed on biopsy (138).

Although numerous studies have evaluated the progression of liver disease in patients superinfected with HDV, most have been done in patients with biochemically proven or biopsy-proven chronic liver disease. Very few prospective, population-based cohort studies have examined this question. A population-based cohort study of 216 Yucpa Indian HBV carriers in Venezuela who were monitored for 5 years provides the best evidence for rapidly progressive chronic liver disease. Among persons with HDV infection, 56% had moderate to severe chronic liver disease at the end of 5 years compared with 0% of persons with HBV infection alone (88).

The results of seroprevalence studies of HDV infection in patients with chronic active hepatitis or cirrhosis are consistent with this observation and indicate that HDV infection is more frequent in this patient population than in asymptomatic HBsAg carriers or those with chronic persistent hepatitis (51, 52, 81, 159, 163, 214). The younger age of patients with HDV infection and chronic hepatitis compared with ages of patients with HBV alone also suggests that there is more rapid progression of disease in this group.

Recently, Govindarajan et al. (83) described five patients with chronic HDV infections who had repeated episodes of liver injury associated with serologic evidence of increased HDV replication. Elevated levels of liver enzymes were related to increases in anti-HDV IgM, HDAg, and HDV RNA. These episodes may be multiple and may last several weeks to months. It has been suggested that these episodes of reactivation, similar to reactivation of chronic hepatitis B, may be important in the progression of liver disease in chronic HDV infection (83). More recent data on 46 patients with chronic delta hepatitis who were monitored for a period of 6 to 116 months documented biochemical reactivation episodes in 41% (84). However, in animal inoculation studies, when chronic HBV carrier chimpanzees that had cleared an initial infection with HDV were reinoculated with a homologous strain of HDV, low-level serum HDV RNA and transient, mildly elevated levels of ALT reappeared. These data suggest the possibility of reinfection with HDV, and some cases of presumed reactivation could be explained by reinfection (141). Further investigation will be needed to explore these possibilities.

Extrahepatic disease in patients coinfected or superinfected with HDV has not been well documented. However, several case reports in the literature have described neurological complications, including Guillain-Barré syndrome and seventh-nerve paralysis with myokomia, associated with acute HDV infection (31, 122). In addition, several studies have described the presence of autoantibodies in patients with HDV infection. Antibodies to microsomal membranes, the basal layer of the rat forestomach, and thymic epithelial cells have been detected, but their clinical significance is unclear (21, 220).

Although there is a well-documented and strong association of hepatocellular carcinoma with chronic HBV infection, this association has not been documented with chronic HDV infection (80, 105).

Recent data from liver transplant patients have shed new light on the conventional wisdom of the pathobiology of HDV infection. Several studies have documented the recurrence of HDV infection in liver transplant recipients without signs of HBV recurrence. Ottobrelli et al. (143) found that HDV recurred in 11 (41%) of 27 transplant patients without HBV recurrence and without clinical liver disease. Of 11 patients who received prophylactic hepatitis B immune globulin, none developed hepatitis B (although several had recurrence of delta hepatitis), while 8 patients who were not given hepatitis B immune globulin developed recurrence of

hepatitis B, which became chronic (143). The return of clinical disease in all cases was preceded by the reactivation of HBV, and when HBV returned, HDAg spread in the hepatocytes. Negro and Rizzetto (140) have suggested that HBV may not be necessary to establish HDV in the graft but may be necessary to activate HDV to disease expression. The liver transplant experience has led to speculation that, in addition to the coinfection and superinfection models of HDV infection, there may be a third pathobiological model, which is HBV superinfection or reactivation of a clinically inapparent HDV infection. The expression of disease in the asymptomatic HDV carrier would then be dependent on superinfection with HBV (140).

PREVENTION AND TREATMENT

Prevention of HDV infection is generally directed at the prevention of acute HDV coinfection and superinfection of HBsAg carriers. Prevention of HDV-HBV coinfection is based on well-known practices that prevent the transmission of HBV. In the hospital setting, universal precautions adapted to prevent exposure to blood and adequate sterilization and disinfection of medical equipment and environmental surfaces are essential components in prevention strategies. In the transfusion setting, routine testing of the blood supply for HBsAg and anti-HBc has significantly reduced the risk of transmission of HBV and HDV. Vaccination of persons at high risk of acquiring HBV will prevent the acquisition of HDV infection. If adhered to, recent recommendations from the Immunization Practices Advisory Committee of the U.S. Public Health Service for the routine vaccination of all infants born in the United States will, it is hoped, significantly reduce the rates of both HBV and HDV infections over the next 20 years (36). Hepatitis B vaccine has also been recommended for all infants in countries of moderate to high HBV endemicity. However, before hepatitis B vaccine can be used in countries with limited resources, the cost of the vaccine must be reduced significantly.

Prevention of superinfection in HBsAg-positive patients is somewhat more difficult, and strategies must be focused on prevention of percutaneous exposure to blood. In areas of high endemicity where poor sanitation and close person-to-person contact have been implicated in transmission, improvement of living conditions offers the only hope of decreasing the rates of HDV infection. HBsAg-positive hemophiliacs who receive pooled plasma products may be at risk of acquiring HDV infection. When possible, blood from single or minipool donors should be used for transfusion (169). HBsAg-positive patients receiving hemodialysis should be screened for HDV if they are considered at high risk for this infection (i.e., intravenous drug users and hemophiliacs). HBsAg-positive hemodialysis patients found to be positive for HDV infection should be dialyzed separately from patients who are HBsAg positive but HDV negative (117).

Treatment of patients with chronic delta hepatitis remains difficult. Early studies indicated that prednisone, azathioprine, levamisole, and adenine arabinoside were ineffective in treating chronic HDV infection (7, 165). Early pilot studies with alpha interferon in doses ranging from 2.5 to 7.5 million units (MU) given for 3 to 4 months indicated that serum HDV levels and disease activity could be reduced but that the beneficial effects were transient and disappeared when treatment was stopped (99, 167, 168, 201). In 1987, a multicenter Italian study was undertaken to determine whether long-term therapy would improve the outcome in patients with chronic delta hepatitis. Sixty-one patients participated in a randomized controlled trial and were assigned to receive either alpha interferon three times a week (5 MU/m^2 for 4 months and then 3 MU/m^2 for an additional 8 months) or no therapy. All patients were monitored for 12 months after treatment. Twenty-six percent of the treated patients achieved a complete response at 12 months, but the rate of HDV inhibition measured as the decrease in serum HDV levels was similar among cases and controls (166). Overall, 25% of the patients treated had some improvement in hepatic inflammation, but liver function studies returned to baseline or increased in all but one of the initial responders (166). Similar results have been recorded by other investigators (150). Preliminary data from a study in Italy indicated that interferon at 9 MU thrice weekly for 12 months resulted in sustained normalization of liver function studies and histologic improvement in 45% of patients. The response was significantly lower in patients treated with 3 MU of interferon for the same time (65). To date, no laboratory or histological parameters can identify those patients who might respond favorably to interferon treatment, and no alternatives to interferon therapy are currently available. One current approach to therapy of patients with moderate to severe chronic delta hepatitis is to use alpha interferon in doses of 4 to 5 MU daily or 9 MU thrice weekly. Interferon should be stopped when no improvement in ALT level occurs within 3 months; if improvement occurs, treatment should continue for 1 year. If HBsAg becomes undetectable, relapse is unlikely, and therapy can be stopped. In patients who have persistent HBsAg, therapy should also be stopped, and the patients should be monitored. Retreatment can be initiated in the event of relapse of disease (100).

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